HIV in infected lymph nodes

SIR — To answer questions raised by Duesberg[1], and in a separate and scientific sense by Phillips *et al.*[2], concerning the quantities of HIV RNA (detected by *in situ* hybridization) contained in the germinal centres of lymph nodes of HIV-infected individuals, we have constructed a test object containing a known number of HIV virus particles in a fibrin clot[3]. This sample can be processed in a manner identical to infected tissue specimens and examined histologically after *in situ* hybridization using a ^{33}P-labelled HIV antisense riboprobe cocktail representing about 9 kilobases of the HIV-1 genome[4]. An equivalent-sense probe control is used to subtract background from consecutive sections of the test object and of the tissues. Tissue specimens are digested with protease to remove proteins adherent to the viral particles[5]. The amounts of hybridization are detected by phosphorimaging the microscope slides in a Fuji BAS 2000 instrument.

Calculations show the test object to contain 15,450 viral particles per mm^2 of a 6-μm section. Based on a comparison of 1-mm^2 (6,000-mm^3) portions of the test object with equal areas of lymph-node germinal centres showing maximum intensity, we estimate the number of viral particles in a germinal centre to be as much as 2.48×10^4 particles per mm^2. Because about 20% of the volume of lymph nodes is occupied by germinal centres in HIV-infected individuals (C.H.F., unpublished data), an entire lymph node may contain of the order of 1.2×10^9 viral particles per cm^3. The amount of viral RNA appears to remain relatively constant in the germinal centres of lymphoid tissues in sequential biopsies. We have examined more than 300 tissues from individuals who had been infected for different periods of time (including gut, spleen and even lung when lymphoid aggregates occur there), which range from the time antibodies develop against HIV until up to 8 years after infection or until loss of germinal centres in the late stages of AIDS. A similar circumstance occurs in other primates infected with SIV.

In reply to questions regarding the infectious nature of such viruses, we refer readers to a series of relevant papers by the late Albert Sabin[6], in which he reported experiments showing that viruses that had been reacted with antibodies retain their infectivity in tissues. It seems reasonable to us that CD4-expressing T lymphocytes become infected following collision with stored, protein-cloaked virus, as the cells traffic through lymphoid germinal centres. There may also be loss of CD4 expression when infected T cells express HIV. Projected over years, the rate of T-cell replacement falls behind their loss and, combined with alterations of cell populations and the developmental microenvironment of the germinal centre arising from the infection, the balance of both structure and function in the immune system is catastrophically altered. We believe that the protein-cloaked virus in germinal centres, combined with some element of follicular dendritic cell function[7] and T-cell kinetics, is the explanation for the extremely slow but inexorable progression of the primate lentiviral diseases.

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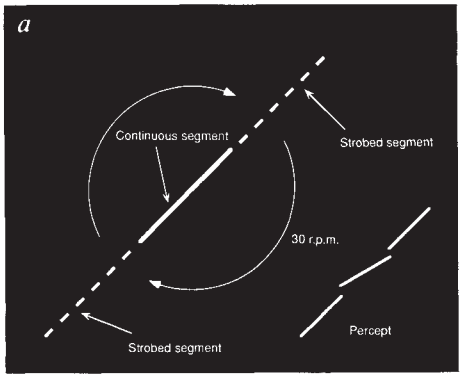
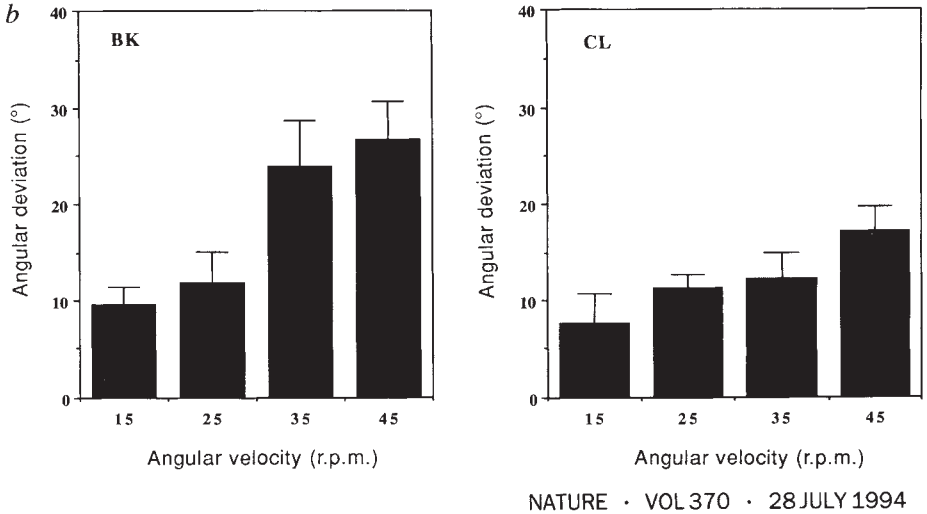
Motion extrapolation in catching

SIR — Many studies investigate how observers might compensate for the latency due to bodily action while executing behaviours such as catching a ball. But success in such interceptive behaviour depends on the observer having accurate information about the ball's initial location. This raises a question that has received surprisingly little attention. How does the visual system compensate for the delay in the transmission of motion information from photoreceptors to 'higher' visual areas of the brain? The problem is serious because the typically estimated delay (τ) of about 100 ms (ref. 1) would cause an object moving at 30 m.p.h. to appear retarded by 4.4 feet in its trajectory!

An effect first observed by MacKay[2] and later rediscovered in our laboratory[3] in the following form, suggests an answer. A single white line is rotated against a black background (see *a* in the figure). The line consists of one continuously illuminated segment and two strobed segments. Observers report a compelling





a, The stimulus was a single physical slit (3.9° long) rotating continuously at 30 r.p.m. against a dark background. The central 1.3° of the slit was continuously illuminated (solid line) and the two outer 1.3° segments were strobed (dashed lines) for 5 ms. Ten observers (six naive) reported a compelling spatial lag of the strobed segments (percept). *b*, The spatial lag increased as a function of the angular velocity of the slit. This lag was measured by having two observers (BK and CL) turn a disk to rotate the strobed segments relative to the continuous segment until they appeared to neither lag nor lead.

spatial lag of the strobed segments, which increases with the angular velocity of the line (*b* in the figure).

How might this effect be explained? I believe the answer lies in the predictability of the continuous segment and the unpredictability of the strobed segments resulting in a differential processing delay. The continuous segment is illuminated for a long enough time for it to be represented cortically. Furthermore, as the angular velocity of this segment is constant, there is information at time t as to where the segment will be at $t + \tau$ ms. It is proposed that in order to overcome the transmission delay, an 'early' visual mechanism corrects the spatial lag by extrapolating the moving object's instantaneous location. Thus, the perceived location, which incorporates the input from this mechanism, is closer to the object's physical location than might be expected from neurophysiological estimates[1] of the delay. The perception of the strobed segments is also contingent on the retinal signal triggering a cortical neural representation, but owing to the unpredictability of the stroboscopic event, the visual system cannot overcome the transmission delay in this case.

If an equal delay for both the moving and the strobed segments is assumed, one could account for the present 'flash-lag' effect in terms of visual persistence of the strobed segments for about 100 ms after their off-set, and the 'deblurring' of the continuous segment by the motion system[3,4]. But according to this account, the strobed segments should appear aligned with the continuous segment at the instant of strobe onset. While observers report that the large visible misalignment (of up to 25°) is already present at the instant of strobe onset.

The present findings suggest that in the case of moving objects the visual system overcomes at least some of the transmission latency through extrapolation. Future experiments will reveal the discrepancy, if any, between the extrapolated and physical locations of moving objects. In this context, it is interesting to note that the computed average (angular deviation ÷ angular velocity) of the time delay of the strobed segments is approximately 82 ms, which is not too different from typical estimates of about 100 ms. Thus, the error between the physical location and the extrapolated location may not be very large.

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Ant sex ratios

SIR — Sundström[1] reported that colonies of the ant *Formica truncorum* on islands in southern Finland produce sexual broods with a bimodal distribution of sex ratios. Colonies headed by queens that Sundström inferred had mated multiply (based on intra-colony allele frequencies at 3–4 allozyme loci) rear significantly more males than females, indeed sometimes only males, whereas colonies headed by putatively monogamous queens do the reverse. Sundström argued that sex specializations result from workers first assessing their relatednesses to male versus female brood, relative to average worker-relatedness asymmetries in other colonies of their population, and then adjusting the sex ratio in their own reproductive interests (specializing on females when relative relatedness asymmetry is high, and on males when it is low).

The hypothesis of genetic-relatedness asymmetry[2,3] requires that worker ants: (1) assess the total number of different males with whom their mother mated (which conceivably exceeds the number of patrilines active during any one worker's lifetime) relative to the mean frequency of polyandry in their local population; and (2) recognize the sex of eggs or larvae, and behaviourally bias their colony's sex ratio, for example via neglect or siblicide; but (3) do not distinguish full sisters from half sisters among eggs or larvae (because discriminative nepotism would always result in female-biased broods). Because Sundström[1,4] provided no evidence that *F. truncorum* workers are altering their queen's preferred sex ratio, it is not possible to judge the plausibility of the mechanisms underlying her interpretation.

An alternative hypothesis to explain bimodal sex ratios in ants[2] is that queens themselves sometimes lay predominantly male (haploid) or female (diploid) eggs, with the workers caring for all the brood. This hypothesis implies synonomy of queen and worker sex-ratio preferences, a possibility that has apparently not been investigated for any species. A direct test would be to compare primary versus secondary sex ratios — that is, samples of reproductive-destined eggs versus alates, controlling for workers' elimination of inviable eggs.

Queens and workers would benefit from manipulating the sex ratio in that outbreeding would be optimized. Colonies of social insects are frequent targets of debilitating parasites and pathogens[5,6], and dispersal from infected areas and genetic variability among progeny might thwart such biotic enemies. Multiple queens, multiple mating by queens and outbreeding all enhance genetic variability[5,6]. Male-biased broods promote both extreme dispersal and outbreeding, especially since male ants are typically much smaller and lighter than reproductive females.

We suggest that queens adjust their mating frequency and, in collaboration with workers, their progeny sex ratio in response to the local severity of parasites and pathogens. If this hypothesis were correct for Finnish *F. truncorum*, queens from islands where diseases are prevalent, where there are local pockets of disease within islands, or where there are survivors from individual diseased colonies, would mate multiply and produce male-biased, dispersive broods. Queens from relatively disease-free localities (for example where the success of daughter colonies is high), in contrast, would mate monogamously and produce female-biased, philopatric broods. This suggestion is an alternative explanation for Sundström's[1] fascinating results.

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SUNDSTRÖM REPLIES — Sherman and Shellman-Reeve offer constructive suggestions for further investigation, together with some new ideas on causes of sex ratio specialization in ants. I agree that queens may gain at least partial control by adjusting the primary sex ratio, and this is clearly the next question to address. In colonies headed by a multiply mated queen, they propose that workers and queen mutually agree to produce a male-biased sex ratio. This is also fully consistent with worker control according to current sex ratio theory, because the optimal sex ratio for workers and queen converge in colonies headed by a multiply mated queen[2,7,8].

However, for the colonies headed by a singly mated queen, Sherman and Shellman-Reeve make some predictions which are not supported by the data. First, if females disperse less than males, as they suggest, local resource competition among females would ensue, with selection for male-biased population-level sex ratios[9]. This stands in contrast to my results, which demonstrate a female-biased population-level sex ratio. Moreover, under local resource competition only small colonies may be expected

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OCCURRENCE OF BAND 3 DELETION AMONG CHILDREN IN THE MADANG AREA OF PAPUA NEW GUINEA

	Cerebral malaria (CM) $n=35$		Uncomplicated malaria (UM) $n=57$		Healthy individuals (HI) $n=103$
Demographics and parasitology					
Mean age in years (s.d.)	4.2 (2.7)	<0.001*	3.5 (1.9)	<0.001†	5.8 (2.1)
Range	1–10		0.8–11		2–11
Area: North (%)	19 (54) in 12 villages	0.20*	35 (61) in 22 villages	0.02†	43 (42) in 11 villages
South (%)	16 (46) in 13 villages		22 (39) in 15 villages		60 (58) in 10 villages
Mean $\log_{10}$ (*P. falciparum* +1) (parasites per μl (s.d.))	4.05 (1.25)	<0.001*	4.09 (0.68)	<0.001†	2.04 (1.71)
Gene frequency and morbidity association					
Band 3 deletion prevalence rate and CI	0% 0–10%		8.8% 3–19%		14.6% 8–23%
Odds ratio, CI and *P* value					
Unadjusted	0 0–0.75	0.01*	0.56 0.15–1.76	0.33	1
Adjusted for age and geographical area	0 0–0.21	<0.001*	0.26 0.06–0.94	0.04	1

Only children living in villages are included. Those living in towns or in suburban areas have different ethnic origins, with known differences in prevalence of the deletion. CM, patients recruited at the Madang General Hospital, defined according to published criteria[10], with a slight modification of the Blantyre coma score[11]. UM, children who attended health facilities in the same areas with a recent history of fever, no other major symptoms or signs, and with a confirmed *P. falciparum* asexual blood stage parasitaemia. HI, children who had not complained of symptoms during the previous week. They were included irrespective of their parasitological status. Deletion in the erythrocyte band 3 gene was determined using polymerase chain reaction (see, for example, ref. 12). The strength of association between the band 3 deletion and disease severity was assessed by comparing the prevalence rate of the red-cell variant gene in CM cases and in HI using Fisher's exact test (two-tailed), and by the exact 95% confidence interval (CI) for the odds ratio. UM and HI were also compared. Adjustment for age and area was made by logistic regression. A quadratic effect of age was fitted because of a significant departure from linearity. Likelihood-based confidence intervals were calculated. * *P* value for CM against HI; † *P* value for UM against HI.

in South-East Asian ovalocytosis is speculative. It is known that ovalocytes are relatively resistant to invasion[5,6] and that they provide some protection against parasitaemia[3]. It seems likely from this study that ovalocytosis might protect against uncomplicated morbidity from malaria to about the same extent as it protects against parasitaemia. Cerebral malaria is a potentially fatal disease, so the frequency of the band 3 deletion in these patients can be taken as an indicator of its selective advantage. We could find no heterozygous individuals among the cerebral malaria cases; ovalocytosis would thus seem to protect from death by malaria.

The view that ovalocytosis is maintained in the population by an advantageous effect operating solely against parasite invasion is questionable. Experimental observations suggest that band 3 deletion plays a specific role in the pathogenesis of cerebral malaria through decreased cytoadherence of infected red cells to cerebral microvessels. This process follows a modification of the band 3 protein in infected red cells and cytoadherence can be blocked in mice with polyclonal antiserum against a polypeptide corresponding to the human band 3 residues 546–553 (ref. 7). Unlike the case of thalassaemic and HbS-containing red cells, previous studies, including samples from the Madang area, have not supported a modifying role of ovalocytic cells on the rosetting ability of red cells[8]. It seems unlikely, therefore, that the protection against cerebral malaria conferred by ovalocytosis in Papua New Guinea operates through the rosetting mechanism.

In screens of around 2,000 subjects (including heterozygous matings) (C.S.M. *et al*, unpublished observations), we have found no individuals homozygous for band 3 deletion. It is thus likely that homozygosity is lethal *in utero*. Ovalocytosis in Papua New Guinea may not be a balanced polymorphism. However, if we assume an equilibrium and that all homozygotes for the band 3 deletion die *in utero*, a prevalence of 15% heterozygosity in the general population, as we have in this study, would imply that 9% of all homozygotes without the gene variant die of malaria before reproduction[9].

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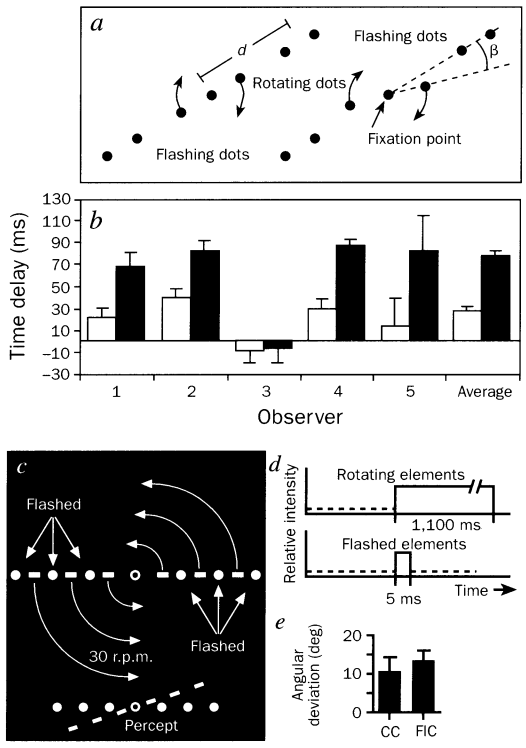
Extrapolation or attention shift?

SIR — Interceptive actions, such as hitting a moving target or catching a ball, need to be adequately timed in order to be successful. Because there is a significant time delay in the transmission of information along the visual pathways, there could be a critical difference between the perceptual and actual position of a moving object. Nijhawan[1] proposed that a moving object is seen closer to its actual physical location, extrapolated from features of the motion, partially compensating for the delay in visual processing.

Most observers report a perceptual misalignment between two sets of aligned dots when one set is in motion (*a* in the figure). Two outer pairs of dots, diametrically opposed to each other, are momentarily flashed in perfect alignment with an inner pair of continuously rotating dots. The moving dots, which are generally seen ahead of the flashing dots, would have their position, according to Nijhawan[1], perceptually extrapolated by about 80 ms.

We interpret this effect as resulting from a longer time delay involved in the visual processing of the flashing dots, rather than an extrapolation of the moving dots. In a series of experiments to investigate attentional mechanisms, we found a clear dependence of this effect on the location of the flashing dots. The magnitude of the perceived misalignment increased as the flashing dots were moved away from the fixation point (*b* in the figure).

In further, but still preliminary, experiments with two other observers, using a similar set-up, we also examined the case in which the positions of the flashing and moving dots were switched, as well as the the original experiment. In all situations, the moving dots were perceived as located ahead of the flashing dots. However, in the switched version of the experiment, the mean angle of perceived misalignment showed a much smaller, or no, dependence on the location of the moving (outer) dots. Furthermore, its magnitude was comparable to those obtained when the flashing (outer) dots were closer to the



Experimental design and results of: *a, b*, Baldo and Klein, and *c–e*, Khurana and Nijhawan. *a, Left*, the stimulus, a pair of dots 1.3° apart in the visual field, rotating at 25 r.p.m. about a central dot (fixation point). Two pairs of dots were flashed in perfect alignment with the central and rotating dots. Distances, *d*, from the central dot to the midpoint between each pair of flashing dots, used in our tests, were 1.45° and 4.74°. *Right*, The situation as reported by most observers: the rotating dots are seen ahead of the flashing dots. The perceived misalignment, β, was assessed by letting the observer adjust the flashing dots until they appeared in alignment. *b*, The misalignment as a time delay and the mean time delay from five naive observers measured at two different distances (data averaged over all observers and weighted by inverse variance). Two other observers, under a similar experimental set-up, offered similar results concerning the original experiment (average time delays: 33 ± 9 ms and 83 ± 7 ms for closer and farther flashing (outer) dots, respectively). A switched version of the experiment was also used where the outer dots moved and the inner dots flashed. The perceptual effect was qualitatively the same, but the strong dependence on the location of the outer (moving) dots was not observed (average misalignment: 24 ±14 ms and 19 ± 9 ms for the closer and farther locations, respectively). These values are comparable to those found in the original experiment, when the flashing (outer) dots were closer to the fixation point. ❑, 1.45°; ■, 4.74°. *c*, The observer fixated the central dot while attentively tracking[6] a line (length = 6.9°) composed of 6 rectangles rotating at 30 r.p.m. A horizontal line (length = 7.8°), composed of 6 circles interleaving the rotating line, was flashed for 5 ms. Observers reported the perceived relative positions of the two lines by varying the angle between two adjustable lines. A strong flash-lag effect was reported (percept). *d*, The intensity profiles of the rotating and flashed elements as a function of time in the FIC condition. *e*, Of 10 observers, 5 viewed the FIC condition first and the complete cycle (CC) second; the order was reversed for the remaining five. A paired *t*-test showed no significant difference between the angle means for the CC and FIC conditions; mean FIC − mean CC = 2.8, $t(9) = 1.70$, $P > 0.10$. The average ($n = 10$) angular deviations for the CC and FIC conditions yield time delays of 58.61 and 74.17 ms, respectively.

fixation point in the original experiment (see figure legend).

These findings support the idea that the perceptual effect is mainly involved in the location of the flashing dots in the visual field. We hypothesize that some amount of time, dependent on eccentricity, is required to bring the flashing dots to a sufficiently high level of sensory awareness for a 'snapshot' of the moving dots to be taken. Such a time delay would be related to the abrupt onset of the flashing dots and might involve attentional mechanisms, either in capturing attention[2] or in shifting the focus of attention from one place to another across the visual field[3,4].

Purely sensorial mechanisms, operating preattentively and depending on eccentricity, cannot yet be discarded. More experiments are needed to distinguish between these possibilities, but we believe that an attentional hypothesis should be examined further.

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KHURANA AND NIJHAWAN REPLY — Baldo and Klein's hypothesis involves the 'shift' or 'capture' of attention from moving elements to flashed elements of abrupt onset. We used two experimental manipulations to distinguish between this and the hypothesis that the lag of predictably moving objects is 'corrected' by the visual system through extrapolation[1], or through some form of neural facilitation applied along the inferred trajectory of moving objects. Neural responses for inferred motion, where an explicit motion signal from the retina is absent, have been observed[5]. Flashed objects, on the other hand, are unpredictable and subject to expected neural delays, which cause their apparent lag.

We addressed the 'attention shift' hypothesis by spatially interleaving the moving and flashed elements. In this condition, observers attentively tracked[6] a rotating line composed of six rectangles. A horizontal line composed of six circles was flashed for 5 ms (*c* in the figure). As the flashed elements occupy the spaces between the attended rotating elements, attention shifts should be negligible and the flash-lag effect should not be observed. However, this display produced a strong effect (*e* in the figure).

Delays due to 'attention capture' were tested by the abrupt onset of both the moving and flashed elements. The display was modified such that the flashed and rotating elements came on simultaneously for 5 and 1,100 ms, respectively. In this 'flash-initiated' cycle (FIC), the rotating and flashed elements have an equally abrupt onset (*d* in the figure), and thus both capture attention equivalently. If delays due to attention capture cause the effect, then none should be observed in this condition. The effect we found, however, did not significantly differ in strength from that observed in the 'complete' cycle (*e* in the figure). When flashed and moving objects are equated in terms of the shift-time or capture-time of attention, observers continue to report the flash-lag effect.

We explain the FIC results on the basis of parallel processing in the visual system[7,8]. The neural processing of both the rotating and the flashed lines begins simultaneously, but the observer does not perceive either stimulus for approximately 100 ms (ref. 9). During this time, the retinal image of the line rotating at 30 r.p.m. has moved through 18°, triggering a motion signal in the magnocellular stream. We suggest that lag-correction, which probably occurs in the fast magnocellular stream, is implemented within that period. The correction process is complete within the time window required for neural signals to yield visual awareness, and before the onset of attentional processing[10].

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